REMARKS

The amendments to claims 1 and 10 are supported in the specification and the term "replication-restricted" is defined at page 11, line 34 through page 12, line 8 of the instant specification. The relevant portion of the disclosure is provided here:

> "Replication-restricted" as applied to a vector of the invention means the vector replicates better in a dividing cell, i.e. either a neoplastic cell or a non-neoplastic, dividing cell, than in a cell of the same type that is not neoplastic and/or not dividing, which is also referenced herein as a normal, non-dividing cell. Preferably, a replication-restricted virus kills at least 10% more neoplastic cells than normal, non-dividing cells in cell culture monolayers of the same size, as measured by the number of cells showing cytopathic effects (CPE) at 5 days p.i. More preferably, between 25% and 50%, and even more preferably, between 50% and 75% more neoplastic than normal cells are killed by a replication-restricted virus. Most preferably, a replication-restricted adenovirus kills between 75% and 100% more neoplastic than normal cells in equal sized monolayers by 5 days p.i.

The amendment to claim 21 is supported in the specification and the term "replication-defective" is defined at least at page 11, lines 30-33. The relevant portion of the disclosure is provide here below:

> "Replication-defective" as applied to a recombinant virus means the virus is incapable of or is greatly compromised in, replicating its genome in any cell type in the absence of a complementing replication-competent virus. Exceptions to this are cell lines such as 293 cells that have been engineered to express adenovirus E1A and E1B proteins.

REJECTIONS UNDER 35 U.S.C. 112 ¶ 2

Claim 1 is rejected as being indefinite, especially in the use of the terms "replication-competent" and the indefinite article "an" in the phrase "which overexpresses **an** adenovirus death protein." However, figure 6 and example 3, page 26, line 21 through page 27, line 17 of the specification describe the recombinant adenovirus vector replicating in neoplastic cells but not in quiescent or non-neoplastic cells. For those vectors encompassed in claim 1 that contain ADP but may not consist of an adenovirus, the specification describes at page 15, lines 18-35, especially at line 22, that "expression of one or more vector proteins essential for replication can be placed under the control of the promoter for a cellular gene whose expression is known to be upregulated in neoplastic cells. Examples of such genes include but are not limited to: the breast cancer markers mammaglobin (Watson et al., *Oncogene 16*:817-824, 1998); BRCA1 (Norris et al., *J. Biol. Chem. 270*:22777-22782, 1995) and *her2/neu* (Scott et al., *J. Biol. Chem. 269*:19848-19858, 1994); and prostate specific antigen (U.S. Patent 5,698,443); surfactant protein B for lung alveoli (Yan et al., *J. Biol. Chem. 270*:24852-24857, 1995); factor VII for liver (Greenberg et al., *Proc. Natl. Acad. Sci. USA 92*:12347-12351, 1995); and survivin for cancer in general (Li et al., *Nature 396*:580-584)." Applicants therefore argue that the specification adequately describes replication-competent vectors that are not recombinant viruses.

Furthermore, applicants argue that the language of claim 1 reflects multiple adenovirus death proteins (ADP) because there are, in fact, multiple isoforms of ADP. The specification, at page 2, lines 28-31, describes ADPs derived from adenovirus types 1, 2, 5 and 6. Hence, the use of the indefinite article is clear and fully supported by the specification.

Claims 1 and 10 (and dependent claims) are rejected as being indefinite in the recitation of the term "overexpresses". The Patent Office has apparently equated the term "overexpression" with the natural level of ADP expression, which reaches a maximum at the late stage of infection. The term "overexpression", which is explicitly defined in the specification at page 12, lines 18-23 (reproduced below), means expression that is above natural, wild-type expression.

> "As applied to recombinant Ad and AAV vectors, the term "overexpresses ADP" means that more ADP molecules are made per viral genome present in a dividing cell infected by the vector than expressed by any previously known recombinant adenoviral vector or AAV in a dividing cell of the same type," (page 12, lines 18-23).

Furthermore, the specification, at page 12, line 33 through page 13, line 8, describes in detail how an adenoviral vector that overexpresses ADP is constructed. For example, "any type of deletion in the E3 region that removes a splice site for any of the E3 mRNAs will lead to overexpression of the mRNA for ADP, inasmuch as more of the E3 pre-mRNA molecules will be processed into the mRNA for ADP. Other means of achieving overexpression of ADP in Ad vectors include, but are not limited to: insertion of pre-mRNA splicing and cleavage/polyadenylation signals at sites flanking the gene for ADP; expression of ADP from another promoter, e.g. the human cytomegalovirus promoter, inserted into a variety of sites in the Ad genome; and insertion of the gene for ADP behind the gene for another Ad mRNA, together with a sequence on the 5' side of the ADP sequence that allows for internal initiation of translation of ADP, e.g. the Ad tripartite leader or a viral internal ribosome initiation sequence." The skilled artisan would recognize that overexpression, as herein defined, means the expression of ADP *beyond* what is normally expressed, both early and late in the infection cycle.

Claims 2 and 11 are rejected on the basis of having unclear language. Claims 2 and 11 are herein amended to clarify the fact that the amino acid limitations (amino acids 1-26, 41-59 and 63-70) are directed to SEQ ID NOS:5, 6, 7 and 8.

Claims 5 and 15 are rejected on the basis of being indefinite. Claim 5 is amended to reflect the fact that SEQ ID NOS:3 and 4 refer to the recombinant adenovirus. Claims 5 and 15 are given their broadest scope and therefore refer to further limitations on the adenovirus component of the recombinant vector, which is a limitation on the recombinant vector of claim 1, which encompasses plasmid DNAs containing said sequences. SEQ ID NOS:3 and 4 are mutant forms of human adenovirus type 5 genomes, *i.e.*, strains GZ1 and GZ3, respectively, as described on page 15, lines 15-17 and Table 1. The only way that claims 5 and 15 may be interpreted is as a replication-competent DNA molecule that contains either Ad5 strain GZ1 or Ad5 strain GZ3.

Claim 10 is also rejected on the basis of being indefinite. Claim 10, in its amended form, clearly refers to a vector that overexpresses the death protein *and* is replication restricted to a neoplastic cell. The neoplastic cell is contacted with the adenoviral vector. The skilled artisan understands that adenoviruses, once placed in contact with a cell, are capable of transduction into the cell. Furthermore, vector DNA which is not a virus, when placed in contact with the cell, is able to transfect into the cell, albeit at a lower frequency of transformation.

Claim 13 is herein amended according to the suggestions of the examiner, and therefore should now be in a condition for allowance.

Claim 14 clearly and distinctly adds an additional step to the method for promoting cell death of a neoplastic cell (claim 10). That additional step being the passive immunization of the patient against the recombinant adenovirus. It is stated in the specification, on page 6 at lines 2-4, "that a recombinant adenovirus ... will stimulate infiltration of inflammatory and immune cells into a tumor treated with the adenovirus and that this host immune response will aid in destruction of the tumor as well as tumors that have metastasized." Therefore, it is expected that supplementation of the patient's immune system with gamma globulins directed against the recombinant adenovirus will further aid to destroy adenovirus infected, ADP expressing neoplastic cells. Applicants argue that the claim is definite and clear, and therefore proper.

Claims 13, 14, 15 and 24 are rejected on the lack of antecedent basis for the limitation "the recombinant adenovirus." Claim 13 is amended to replace the term "the recombinant adenovirus" with the term "vector", which is fully supported by the base claim number 12. Claim 13 and those claims that are dependent on claim 13 should now be in a condition for allowance.

Claim 21 is amended to clearly define the scope of the claim to encompass more than one different and distinct type of vector containing recombinant adenoviruses. This amendment is supported by the specification on page 7, lines 19-21 and 26-28.

Claim 24 is rejected for being indefinite in the use of the phrase "complements spread of the replication-defective adenovirus tumor." The Patent Office states that the "structural relationship between the replication-competent and the replication-defective adenoviruses or what definition or the metes and bounds apply to the term 'complements' in the context of the claim" are unclear. Applicants respectfully point out that the specification, at least at page 17, line 22 through page 18, line 2, explicitly defines the underlying mechanism behind the claimed invention, thereby shedding considerable light on the claim. The specification tells us that, by combining replication-defective viral vectors expressing an anti-cancer gene product with replication-competent viral vectors described herein, it is expected that the result will be amplification and rapid spread of both vectors to surrounding cells. The idea being that the replication-competent virus provides the replication proteins, for example the E1A protein, in trans to the replication-incompetent virus (*e.g., Δe1A*) genome, thereby providing genetic *complementation* to the mutant virus genome. Although the concept of "complementation" is art recognized, in view of the potential for misunderstanding, claim 24 is herein amended to contain the term "facilitates" in place of "complements." The term "facilitates" does not constitute new matter, since it explains the effect that is derived from co-transformation of a vector that is replication-restricted to neoplastic cells and a vector that is replication-defective. In view of the arguments and amendment presented above, claim 24 should now be in a condition for allowance.

CLAIM REJECTIONS UNDER 35 U.S.C. 112 ¶ 1

Claims 1-5, 10-15, 20-22 and 24 are rejected under 35 U.S.C. 112 ¶ 1 as not being enabled by the specification. The crux of the arguments presented by the Patent Office are that the utility of the invention is in its use as an anti-cancer therapy and that the art of gene therapy is underdeveloped and unpredictable.

The Office cites W. French Anderson as stating that gene-therapy is plagued with problems of poor gene delivery, poor gene expression, immune system interference and inefficient manufacture of vectors. The Office further cites Verma and Somia as stating that the major problems with gene-therapy are the ability to *efficiently* deliver genes and to obtain *sustained* expression. The Office then goes on to cite Curiel as stating that the problems with adenoviral vectors include widespread tropism beyond the

site of injection (a problem which the instant invention nicely circumvents) and the need for developing cell-specific targeting properties.

Applicants point out that the utility of this invention extends beyond treating tumors in a patient. In addition to disclosing the *in vivo* treatment of tumors, the specification discloses the transfection of tumor cells, *in vitro* or *ex vivo*, with vectors that overexpress ADP. The invention therefore has utility in the selective destruction of cells *ex vivo*. This is useful for cell therapy procedures, such as bone marrow transplantation, wherein cancerous cells can be selectively eliminated from the cultures prior to or concomitant with implantation into the host. Therefore, it is improper to limit the entire invention only to *in vivo* tumor treatment, which is claimed in claim 13 and dependent claims. It is also a routine matter for the skilled artisan to create a vector comprising an ADP gene, which is disclosed in the instant specification, under the control of a cancer cell promoter, which is also disclosed in the instant application, and to transfect that plasmid into cells. (See discussion above regarding cancer cell promoters.)

Claim 13, as herein amended, is not drawn to classical gene therapy *per se*, but rather to treating a tumor with a vector comprising a recombinant adenovirus, which has been mutated to exhibit deficient replication in non-neoplastic cells, while allowing replication to occur in neoplastic cells. The "methods-of-treating a tumor" claims (13-15, 20-22) involve contacting a tumor with the recombinant adenovirus, wherein the adenovirus is **not** designed to deliver a heterologous (or non-adenoviral) gene into a cell, but rather to overexpress a subset of adenoviral genes, *e.g.*, ADP. Subsequent to infection of neoplastic cells, the adenovirus replicates, overproduces ADP, and leads to the selective destruction of those neoplastic cells. The adenovirus is **not** required to deliver a heterologous gene, nor is sustained expression of the ADP gene required, because the cells in which expression of ADP occurs are destroyed. Therefore, the invention is not subject to the same requirements of long term gene therapy, in which a heterologous therapeutic gene, such as the CFTR gene, for example, is delivered to a cell to cure a genetic defect.

The patent office cites the Curiel review as teaching that "widespread tropism" of adenoviral vectors away from the cite of injection is a limitation to this approach to tumor ablation, leading to inefficient gene transfer. However, applicants note that the Curiel reference also teaches straightforward methods of targeting adenoviral vectors to specific types of tissues or tumor cells. Curiel teaches us (p. 163, 2nd full paragraph) that it is possible to target specific tissues, *e.g.*, vascular smooth muscle cells and endothelial cells using a bispecific anti-FLAG/anti-integrin approach or a FGF2 retargeting approach. On page 165, in the second full paragraph, Curiel emphatically states that "[m]odification of tropism was

successfully achieved, indicating that true cell-specific delivery is possible." Applicants argue that this statement published by a skilled artisan supports the use of adenovirus vectors to deliver therapeutic gene products to specific tissues, including tumors.

The Patent Office further cites Gomez-Navarro reporting that "transduction efficiencies of *presently available* vectors have been shown to be inadequate ..... [and] it has not been possible to *modify* a sufficient number of tumour cells to achieve a clinically relevant tumoral response." Again, the Patent Office is assuming that the vector embodiment of the present invention (claim 13), which is shown in the instant specification to infect tumor cells in an art recognized *in vivo* model, is used to deliver a gene for either compensation for an endogenous gene mutation or molecular chemotherapy using HSV-thymidine kinase. The Gomez-Navarro reference teaches us that it is crucial to deliver the optimal number of virions to the correct cell-type so that therapeutic gene expression is optimal to that cell-type and limited only to that cell type. The present claims, as herein amended, are limited to vectors which are replication-restricted to cancer cells. Furthermore, the tumor treatment claims are further limited to vectors which are adenoviruses and which are fully supported by the disclosed examples. The recombinant adenoviral vector of the instant invention is able to be administered at lower, non-toxic doses at the tumor site. Upon transduction into the cells, the vector is able to replicate only in the tumor cells and is therefore *self-limiting* to only neoplastic cells. If "leakage" of the vector occurs into neighboring tissues, the vector, which does not encode HSV-tk or other chemotherapeutic genes, can not replicate and hence can not spread further. Therefore, applicants argue that the claimed invention addresses the problems outlined by the Anderson, Curiel, Gomez-Navarro, and Verma references, and is therefore not subject to an enablement rejection based on these references.

The Patent Office further argues on pages 10 and 11 of the Office action that the instant invention fails to provide for adequate targeting of the adenovirus to cells of interest and fails to provide sufficient guidance to predict which types of cancers may benefit from such a treatment. As to the first point, the specification at page 18, line 31 through page 19, line 8 describes several modes of administration of the vector to a tumor, including direct intratumoral injection and intravenous, subcutaneous, intramuscular, transdermal, intrathecal and intracerebral injection. The specification provides specific working examples of intratumoral injection at page 27, lines 31-34, page 28, lines 25-27 and page 28, lines31-32, which also describes the administration of defined amounts of adenoviruses to tumors of a defined size. For example, each A549 tumor that is 20 to 50 microliters in size received 3 x 10^8 PFUs and up to 5 injections of 5 x 10^8 PFUs. Hep3B tumors that are 100 microliters were treated twice per week for three weeks with

5 x 10^7 PFUs of vector. In view of this evidence, applicants argue that the specification adequately provides a method of targeting and administering adenovirus vectors to a tumor *in vivo*.

As to the second point, working example 2 describes a simple cell lysis and virus spreading assay, which is used to test the adenovirus vectors against various specific types of human tumor cells. Page 26, lines 4-13 describes the infection and destruction of the following tumor cells: HeLa (cervical carcinoma), DU145 (prostate), and pC3 (prostate) cells at 10^{-2} PFU/cell, ME-180 (cervix) and Hep3B (liver) at 10^{-1} PFU/cell, and U118 (glioblastoma) and U373 (glioblastoma) at 10 PFU/cell. Further, at page 26, lines 28-35 and page 27, lines 3-11, the specification describes normal cells (fibroblasts, bronchial epithelial cells and growing lung endothelial cells) that *survive* the cell lysis and virus spreading assay. Therefore, in the disclosure, the skilled artisan is provided sufficient guidance to test the sensitivity of any and all neoplastic cells to the adenovirus vectors. The viral titer, which is to be administered to the tumor, would be adjusted by the skilled practitioner based upon the results of this simple test. If the cells are not responsive to the adenoviral vector therapy, then the skilled artisan would make a judgement call as to whether or not there will be a therapeutic benefit to using the adenoviral vector therapy. Of course, the skilled artisan would obtain the neoplastic cells from the initial biopsy, or a later biopsy, as the case may be.

The Office argues that the disclosure does not enable the delivery of the adenoviral vector, in view of the problems enumerated by Jain (1998) and Hobbs *et al* (1998), specifically those "multiple barriers limiting delivery of therapeutics to solid tumors..." Jain (1998) teaches about the microvascular architecture of solid tumors and is therefore relevant only to intravenous delivery of the adenovirus vector, and not to direct intratumoral injection. At page 53, column 1, Jain suggests that tumors in non-necrotic regions may be treated intravenously, whereas tumors in necrotic or seminecrotic regions will be less amenable to i.v. delivery of therapeutics, and would therefore be more amenable to intratumoral injection. On page 55, column 1, lines 15-18, Jain teaches that "vascular permeability and hydraulic conductivity of tumors in general is significantly higher than that of various normal tissues." This statement further supports the effectiveness of the instant invention for treating tumors. For example, a "leaky" vessel, which has wide endothelial junctions, will more likely permit the passage of virions into the tumor. Furthermore, it is important to recognize that the teachings of Jain relate more to simple diffusion of molecules through a complex tumor, whereas the instant invention delivers a geometrically replicating, self-limiting infective agent that expands in cancerous cells, lyses those cancerous cells and infects, in larger numbers, neighboring cells. Therefore, the instant invention is not subject to the

limitations of diffusion that are taught by Jain, which teaches nothing about viral spread through a solid tumor.

The Office uses Hobbs et al. (1998) against the instant claims as teaching that variations in vascular pore cut off sizes in tumors are unpredictable and variable. Again, this reference is applicable only to i.v. administration of the adenoviral vector. On page 4608, column 2, under the "Results" section, lines 12-13 and Figure 1, Hobbs teaches that "[t]he majority of tumors exhibited a vascular pore cut off size between 380 and 780 nm." Please note that this statement refers to both cranial and subcutaneous tumors (see Fig. 1 for clarification). The skilled artisan knows that an adenovirus is 70-90 nm in diameter, which is smaller than even the cranial Shionogi tumor cut off size of 100 nm (Table 2, page 4609, column 2.) Therefore, given the fact that the majority of subcutaneous and cranial tumors have a cut off greater that the adenovirus diameter, the skilled artisan would expect that the adenoviral vectors of the instant invention would pass into tumors from the circulatory system. Please note that the hard data presented in Hobbs *et al.* teaches that the blood-brain barrier is effectively *transparen*t in cranial tumors, and therefore cranial tumors are amendable to adenoviral therapy.

The Office misapplies the teaching of Hobbs in the statement on page 12, lines 3-5 of the Office action. Please note that the reference of the drop in pore cut off size to less than 7 nm refers to a specific single testosterone dependent tumor. When testosterone is *withdrawn*, the pore size shrinks. Under "normal" hormonal conditions, the pore size is 200 nm (Hobbs et al., page 4607, column 2, lines 13-17). The skilled artisan would recognize, based upon this observation, that adenoviral therapy may not work well in conjunction with hormone ablation therapy on certain types of tumors. Applicants argue that the teachings of the Hobbs reference are misapplied against the instant application.

The Office argues on page 12, paragraph 2 of the Office action, that the nude mouse model as used in Example 4 and 8 are not sufficient given the unpredictability of the art and the media hype (Fox) surrounding the death Jesse Gelsinger in 1999 from immune system complications after adenoviral gene-therapy. The Office uses Elshami et al. (1996) to argue that the claimed invention should be tested in an immune-competent model, such as the Fisher rat model. Firstly, the Elshami paper does not describe a human tumor model and therefore is subject to very limited interpretation regarding safety and efficacy. The tumor model in this reference is "established from experimentally induced mesotheliomas in Fisher 344 rats exposed to asbestos ... and closely *mimics* human disease" (Elshami, et al., p. 142, col. 2, lines 4-5 under "Cell Line" and p. 142, col. 1, last paragraph; emphasis is mine). By the authors' own admission, the rat model behaves differently than human tumor cells, *e.g.*, at page 146, second paragraph under

"Discussion", lines 3-4, the workers state that "these [rat II-45 mesothelioma] cells were less sensitive to GVC than *human* mesothelioma cells." Furthermore, the system that was evaluated in Elshami is a replication *defective* Adenovirus RSV-tk system, which is not a *replication-restricted* system, which can be delivered to the patient at lower titers. However, in spite of the inappropriateness of the Elshami study regarding genuine human tumors, this reference does show very promising and positive results. For example, Table 1 (p. 145, col. 1) shows significant life extension and reduction in mean tumor size in rats after Ad.RSVtk/GCV treatment. On page 146, col. 2, the authors state in the discussion that "[t]he experiments in this study demonstrate that the Ad.RSVtk/GCV system can effectively treat established tumors within the pleural space of immunocompetent animals." Applicants state, for the record, that it is only necessary to test human adenoviral anti-cancer therapies on genuine human tumor models that are accepted in the art. The art accepted model is the nude mouse, since intact immune systems will not tolerate foreign (*i.e.*, human) cells.

Secondly, the Nature Biotechnology commentary by Fox regarding the death of Jesse Gelsinger is not an appropriate citation against the instant application. The skilled oncologist or gene therapist recognizes that the Gelsinger death was precipitated by excessively large doses of adenovector and the alleged ignoring of clinical data showing problems with the subject during the trials. Furthermore, the gene-therapy trial was not an anti-cancer trial and the vector was replication defective, and therefore was administered to the subject at very high titers. The instant invention is for anti-cancer therapy and involves a replication competent vector that can be administered (as described in the examples) at relatively low titers. Furthermore, applicants respectfully remind the US Patent and Trademark Office, "[t]he Office must confine its review of patent applications to the statutory requirements of the patent law....Thus, while an applicant may on occasion need to provide evidence to show that an invention will work as claimed, it is improper for the Office personnel to request evidence of safety in the treatment of humans, or regarding the degree of effectiveness." (MPEP 2107.02 subsection V.) According to *In re Brana*, "the associated costs [of clinical (human) testing] would prevent many companies from obtaining patent protection on promising new inventions, thereby eliminating an incentive to pursue, through research and development, potential cures in many areas such as the treatment of cancer." (34 USPQ2d 1443). Furthermore, *In re Brana* states that the National Cancer Institute has "explicitly recognized ... murine tumor models as standard screening tests for determining whether new compounds may be useful as antitumor agents (34 USPG2d 1442)."

Still further, there exists a growing body of evidence demonstrating safe and effective treatment in humans using adenovirus-based antitumor agents. Clayman et al, July 1999, demonstrate the safety

and effectiveness of Ad-p53 as a surgical adjuvant (administered via intratumoral injection) in advanced head and neck cancers (Clayman *et al.*, abstract). The safety and efficacy was first demonstrated in a nude mouse xenograft model (p. 1715, col.2, lines 27-35). In another Phase I clinical study, Stewart et al, (1999) demonstrate the safety and effectiveness of a replication defective adenovirus encoding IL-2 (an immune system cytokine). "Local tumor regression at the site of injection [of the adenovirus vector] was noted in six [out of 23] patients " (page 351, col. 2, lines 23-26). Further, on page 358, column 1, lines 6-5, the authors state that "[t]he results of th[e] phase 1 trial further demonstrate that AdCAIL-2 is safe and relatively non-toxic. No evidence for significant vector dissemination, shedding or generation of replication-competent adenovirus was seen." Regarding anticipated immunity problems, the workers state on page 355, lines 1-11, that "an unanticipated persistence of the vector in day 7 or 14 tumor biopsies from 18 of 22 patients (83%) ... suggests that previous immunity to adenovirus infection does not preclude prolonged persistence of adenovectors after a single injection." Furthermore, applicants submit that the AdCAIL-2, by virtue of the fact that it encodes a potent immune-stimulatory cytokine, namely IL-2, closely parallels the claimed invention in its potential to stimulate an immune response (paragraph spanning page 13 and 14 of the Office action), and therefore is anticipated not to "limit the cytolytic spread of the adenovirus replication in tumors." Therefore, the applicants and those skilled in the art have sufficient reason to expect *a priori* that the claimed invention is likely to be safe and effective in an immune competent host.

The fact remains, the instant specification discloses in sufficient and enabling detail the effective treatment of *human* tumors in the closest art accepted murine model that is available (see MPEP 2107.02 III and IV.) Therefore, applicants argue that the claims to tumor treatment are sufficiently enabled by the specification.

The Patent Office notes for the record that "the claimed invention embraces a wide breadth of embodiments for use in the claimed methods that are not accompanied by sufficient guidance" in the specification. The amendment to claim 13 limits the vector to a recombinant adenovirus, for treatment of tumors in a patient. The recombinant adenovirus vectors that are replication-restricted to neoplastic cells were used in the nude mouse studies described in examples 4 (page 27 line 20 - page 29 line 11.) For example, at least at page 27, lines 33-34 of the instant specification, the administration of vectors KD1, KD3, dl309 and (at page 28, line 26) GZ3 to nude mice harboring human tumors shows a significant reduction in the growth of tumors, compared to control vector pm 734.1 administration. Therefore, applicants submit that the invention, as claimed, embraces embodiments that are fully enabled by the specification, and supported by working examples.

Claims 2 and 11 are rejected on the basis that the skilled artisan is not provided "sufficient guidance on how to make and use variants" of ADP. The Patent Office uses an old reference from 1976 to support the charge that, in order to make conserved variants, painstaking experimentation needs to be performed. However, the skilled artisan of 1999 knows the basic structure of α-helices, transmembrane domains, β-sheets, α/β-barrels and other secondary structural motifs and functional domains (*e.g.*, kinase domains). The skilled artisan is able to make simple conserved amino acid changes that have a very low likelihood of disrupting ADP function, and such changes can be made without undue experimentation (see page 13, lines 19-31, of the instant specification, wherein conservative amino acid substitutions are described). According to well-known tenets of protein structure function, the skilled artisan knows how to make conserved amino acid changes to "minimize reorganization of the structure of the [protein], either locally or globally.". (See Fersht, Alan, Structure and Mechanism in Protein Science, Chapter 15 *Protein Engineering*, pp. 425-427. W. H. Freeman, New York, 1998.)

Applicants agree that in 1976, soon after the landmark denaturation/renaturation kinetics studies of Chris Anfinsen, the field of biochemistry did not have the advantage of a huge database of genome and protein sequences with ascribed functions, as well as a growing database of three dimensional structures of proteins. In fact, according to the Protein Data Bank at the Brookhaven National Laboratory, only 58 protein structures were available to the public in 1976 (see tabulation of PDB Content Growth, which is herein enclosed). However, at the time of filing of the instant application in 1999, the National Center for Biotechnology Information's Molecular Modeling Database (MMDB), which contains all of the entries of the Protein Data Bank, contained between 8,942 and 11,364 protein structures. According to Bryant and Hogue (*Structural Neighbors and Structural Alignments: The Science Behind Entrez/3D*, August 1996; paragraph 2, lines 4-6), "[s]ince biological functions are often conserved among members of a homology group, and/or described in the associate Medline abstracts, one may easily explore the structure-function relationships of an entire protein family by traversing these neighboring relationships." What this means is that the protein structure data bank contains a sufficient number of entries to represent most of the protein families found in the publicly available GenBank and/or SwissPro databases. Thus, armed with this mass of data, the instant specification, and the knowledge of simple protein secondary structure relationships, the skilled artisan can easily make ADP functional variants. Importantly, the specification provides descriptions in depth structure-function analyses (page 38, line 2 through page 40, line 18) and of simple cell death assays (page 38, lines 15-17 and page 38, line 33 through page 39, line 26), which can be employed by the skilled artisan to determine ADP activity. Therefore, applicants argue that there is sufficient guidance contained in the specification, in view of the state of the art and the level of skill at

the time of filing of the instant application, for the skilled artisan to make and test ADP variants without undue experimentation.

Claim 14 is rejected on the basis of a lack in "sufficient guidance concerning the purpose of [the passive immunization step]." Applicants herein submit that the skilled artisan understands that antigen bound IgG (gamma-globulins) activate the complement pathway, which leads ultimately to cell lysis. The skilled artisan understands that neoplastic cells treated with the instant adenoviral vector will express viral antigens on their cell surfaces. Exogenously added gamma-globulins, with specific reactivity toward adenovirus, will bind to the transfected neoplastic cells, then activate the classical complement pathway, leading to the ultimate lysis of the neoplastic cell. Chapter 15 of the standard medical text Cellular and Molecular Immunology, by Abbas *et al.*, Saunders, Phila., 1994, is included with this submission. See especially the section entitled *Complement- Mediated Cytolysis*, page 310. In addition, the administration of gamma-globulins is a routine medical procedure and exact titers and administration protocols can be easily formulated by the skilled physician based on, for example age and size of the patient, and the extent of the tumor.

Claim 24 is rejected on the basis of a lack in "sufficient guidance or rationale" for administering to the tumor a replication competent and a replication-defective adenovirus that expresses an anti-cancer gene product. The amendment to claim 1 limits the replication-competent vector to neoplastic cells only, wherein the vector is replication deficient in non-neoplastic cells. As discussed above, the theoretical underpinnings to administering both types of vectors is discussed at page 17, line 22 - page 18, line 22 of the instant specification. Basically, the idea is to be able to administer replication-defective anticancer vectors to the patient at much lower, and hence safer, doses. The replication-defective anticancer vectors will be able to replicate and expand only in those cells that contain replication-competent adenovirus vectors. In other words, the self-limiting replication-competent vector will also function to limit the replication-defective anticancer vector from overt infection of non-neoplastic cells. This is not a mere germ of an idea, but a very valuable, well though-out improvement over current adenoviral anticancer therapies now in clinical trials.

Applicants respectfully request that the rejection of claims 1-5, 10-15, 20-22 and 24 be reconsidered and withdrawn in view of the amendments to the claims and discussion above.

CLAIM REJECTIONS UNDER 35 USC § 102 (b) and (e)

Claims 1-4 and 10-12 are rejected under 35 USC 102(b) as being anticipated by Tollefson et. al., (J. Virol., 70:2296-2306, 1996). Tollefson only describes experiments in which the adenovirus death protein (ADP) is deleted or expressed normally. That paper shows that ADP is necessary for *efficient* cell lysis but is not required for the adenovirus cytopathic effect. Furthermore, that paper describes *dl309*, which, importantly, is ***not*** replication-restricted to neoplastic cells (see Fig. 6 of the instant application). However, the reference absolutely *does not* teach anything about an adenoviral vector that *overexpresses* ADP and/or is replication-restricted to neoplastic cells. Claims 1 and 10 are herein amended to contain the limitation that the vector is replication-restricted to neoplastic cells. This amendment removes the vector *dl309* from the claimed invention.

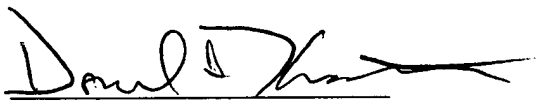
Claims 1-4 and 10-13 are rejected under 35 USC 102(e) as being anticipated by US Patent No. 6,197,293 (Henderson et al., issue date 3-6-01, filing date 3-2-98, priority date 3-3-97). That patent discloses an E3-deleted replication-competent adenovirus vector that expresses various adenovirus genes, including ADP, under the control of prostate-specific control sequences. Specifically, the '293 patent is directed to adenoviral vectors that contain a probasin transcriptional regulatory element to regulate gene expression or replication, and are therefore limited to the dorsolateral prostate and possibly other tissues that are responsive to androgens (see '293 column 5, lines 12-14). The '293 vector can be used to treat prostate tumors. More specifically, the Patent Office points out the CN751 vector, which harbors an E3-deletion and overexpresses ADP. Applicants respectfully point out that CN751 has a wild-type promoter controlling E1A and E1B and is therefore capable of replication in both neoplastic *and* non-neoplastic cell types. CN751 is constructed from a 5' EcoRI fragment of CN702 (which is wild-type Adenovirus type 5; see column 39, lines 61-62 and column 40, lines 1-6 of '293) spliced to the 3' EcoRI fragment of CN252 (which contains the ADP gene under control of the Prostate specific transcriptional response element). Since the replication control region of CN751 is wild-type, CN751 would therefore be expected to replicate in any infected cell, regardless of its status as neoplastic or not, and overexpress ADP only in prostate (or androgen sensitive) cells. Furthermore, the treatment claims of the instant invention are drawn to any and all neoplastic cells, not just to prostate cells (whether or not said prostate cells are cancerous or not). Therefore, in view of the amendments in claims 1 and 10, which limits the vectors to those that are replication-restricted to neoplastic cells, applicants submit that patent '293 does not anticipate claims 1-4 and 10-13 of the instant application. Applicants request that the rejection to claims 1-4 and 10-13 be withdrawn and the claims allowed.

CLAIM REJECTIONS UNDER 35 USC § 103

Claims 1-4, 10-13 and 20-22 are rejected under 35 USC 103(a) as being obvious over the '293 patent in view of Freytag, et al., which teaches that a combination of viral therapy and chemotherapy/ radiation therapy has enhanced cell killing properties. The removal of the '293 patent as a prior art reference under 35 USC § 102 (e), using the arguments discussed above, is sufficient to remove the obviousness rejection. Freytag, *et al.,* disclose the use of adenovectors that are competent to replicate in both neoplastic *and* non-neoplastic cells, but preferentially replicate in cells that are p53 deficient. Recall that p53 minus cells are recalcitrant to radiation therapy. By combining the radiation, chemotherapy and "p53 deficient" targeted suicide gene-therapy, there is thus an enhanced killing. In view of the amendment to claim 21, claims 20-22 are drawn only to vectors that are not competent to replicate in non-neoplastic cells, yet these vectors can replicate in p53 plus cells. Therefore, applicants submit that the instant invention *is not* obvious over Henderson in view of Freytag. Applicants respectfully request that the rejection of claims 1-4, 10-13 and 20-22 be reconsidered and withdrawn in light of the claim amendments and above discussion.

The amendments to claims 1, 2, 5, 10, 11, 13, 21 and 24 are instituted to more distinctly claim and particularly point out the invention. Applicants believe that the claims are in condition for allowance, and respectfully request such action.

Respectfully submitted,



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June 8, 2001

AMENDED CLAIMS

546 C1 1. (Amended) A recombinant vector which is replication-restricted to neoplastic cells and which overexpresses an adenovirus death protein.

B1 2. (Amended) The recombinant vector of claim 1 wherein the adenovirus death protein comprises amino acids 1-26, 41-59, and 63-70 of either SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 or SEQ ID NO:8, or a conservatively substituted variant thereof ; or

wherein the adenovirus death protein comprises SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8.

B2 C2 5. (Amended) The recombinant vector of claim 4, wherein the recombinant virus comprises SEQ ID NO:3 or SEQ ID NO:4.

546 C3 10. (Amended) A method for promoting death of a neoplastic cell comprising contacting the neoplastic cell with at least one vector, wherein said vector is replication-restricted to neoplastic cells and overexpresses an adenovirus death protein.

B3 11. (Amended) The method of claim 10 wherein the adenovirus death protein comprises amino acids 1-26, 41-59, and 63-70 of either SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 or SEQ ID NO:8, or a conservatively substituted variant thereof ; or

wherein the adenovirus death protein comprises SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8.

B4 13. (Amended) The method of claim 12, wherein the neoplastic cell is contained in a tumor in a patient and the contacting step comprises administering the vector to neoplastic cells of the tumor, wherein the vector comprises a recombinant adenovirus.

B5 21. (Amended) The method of claim 20 comprising administering more than one distinct type of recombinant adenovirus to the tumor and treating the tumor with radiation, wherein at least one recombinant adenovirus is replication-defective.

B6 546C5 24. (Amended) The method of claim 13, further comprising administering ~~to the tumor~~ one or more replication-~~defective~~ adenovirus which expresses an anti-cancer gene product, wherein the recombinant adenovirus facilitates the spread of the replication-defective adenovirus in the tumor.